

Attorney Docket No.: 8920-000005

AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

Listing Of Claims

1. (Cancelled)

2. (Cancelled)

3. (Currently Amended) A method for producing an enzyme cellobiase, in the presence of glycosylation inhibitor 2-deoxy-D-glucose from cultures of *Termitomyces clypeatus*, said preparation containing high concentration of enzyme cellobiase in comparison to a control culture, grown in absence of glycosylation inhibitor 2-deoxy-D-glucose, the said method comprising the steps of:

(a) inoculating a mycelial culture of the *Termitomyces clypeatus* into sterilized medium containing carbon and nitrogen sources, inorganic salts, organic nutrients and glycosylation inhibitor 2-deoxy-D-glucose in the range of about 10 $\mu\text{g/ml}$ ~~0.05 $\mu\text{g/ml}$~~ to about ~~2~~ 1 mg/ml at a pH of between ~~about~~ 3 to 8;

(b) growing the mycelial culture at temperatures between 20-37°C under shaking aerobic conditions; and

(c) separating culture medium from the mycelia to obtain the enzyme preparation containing cellobiase activity ~~[[.]]~~ said enzyme having an increased enzymatic activity in the range of about 4-15- ~~2.236~~ units/ml to about ~~140.60~~ 97 units/ml in the presence of glycosylation inhibitor 2-deoxy-D-glucose in comparison to cellobiase activity produced by the same organism under the same conditions in absence of the glycosylation inhibitor 2-deoxy-D-glucose.

4. (Cancelled)

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5. (Cancelled)

6. (Cancelled)

7. (Currently Amended) The method ~~as claimed in~~ of claim 3, wherein the carbon source ~~[[s]] of step (a) are~~ is selected from the group consisting of carbohydrates, agrowastes, TCA cycle acids, amino acids, or and D-glucosamine, wherein the carbohydrates are selected from the group consisting of cellobiose, mannose, fructose, xylose, arabinose, starch, dextrine, cellulose, cotton, and xylan; wherein agrowastes are selected from the group consisting of baggasse powder, rice-straw powder, wheat bran, corn cob powder, and corn powder; wherein the TCA cycle acids are selected from the group consisting of succinate, fumarate, and malate; and wherein the amino acids are selected from the group consisting of aspartate, glutamate, serine, histidine, and alanine.

8. (Cancelled)

9. (Currently Amended) The method ~~as claimed in~~ of claim 3, wherein the nitrogen source ~~in step (a)~~ is selected from the group consisting of ammonium chloride, ammonium nitrate, ammonium dihydrogen orthophosphate, and potassium nitrate.

10. (Currently Amended) The method ~~as claimed in~~ of claim 3, wherein the sterilized medium ~~in step (a)~~ comprises an organic nutrient selected from the group consisting of malt extract, yeast extract, potato extract, peptone, soya-peptone, bacto-peptone, and corn steep liquor.

11. (Currently Amended) The method ~~as claimed in~~ of claim 3, wherein the sterilized medium further comprises a detergent selected from group consisting of Tween-20, Tween-80, and Tween-100.

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12. (Cancelled)

13. (Currently Amended) The method ~~as claimed in claim 8 of claim 3~~, wherein ~~enhanced enzyme~~ activity of cellobiase is about 2.23 units/ml in presence of about ~~0.05 mg/ml~~ of and the 2-deoxy-D-glucose is present at a concentration of about 0.05 mg/ml.

14. (Currently Amended) The method ~~as claimed in claim 8 of claim 3~~, wherein ~~enhanced enzyme~~ activity of cellobiase is about 50.09 units/ml in presence of about ~~1 mg/ml~~ of and the 2 deoxy-D-glucose is present at a concentration of about 1 mg/ml.

15. (Currently Amended) The method ~~as claimed in claim 8 of claim 3~~, wherein ~~enhanced enzyme~~ activity of cellobiase is about 90 units/ml in presence of about ~~1 mg/ml~~ of and the 2-deoxy-D-glucose is present at a concentration of about 300 µg/ml.

16. (Currently Amended) The method ~~as claimed in claim 8 of claim 3~~, wherein ~~enhanced enzyme~~ activity of cellobiase is about 140 units/ml in presence of, about ~~500 µg/ml~~ of the 2 deoxy-D-glucose is present at a concentration of about 1 mg/ml and mannose is present at a concentration of about 500 µg/ml.

17. (Cancelled)

18. (Cancelled)

19. (now) A method for producing cellobiase, said method comprising:
(a) inoculating a mycelial culture of the *Termitomyces chypeatus* into a sterilized culture medium containing carbon and nitrogen sources, inorganic salts, organic nutrients at a pH of between 3 to 8 and a glycosylation inhibitor selected

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from the group consisting of tunicamycin, 1-deoxynojirimycin, and gluconolactone;

(b) growing the mycelial culture at temperatures between 20-37°C under shaking aerobic conditions; and

(c) separating culture medium from the mycelia to obtain cellobiase activity in the range of about 1,2075 units/ml to about 6,1820 units/ml.

20. (new) The method of claim 19, wherein the glycosylation inhibitor is tunicamycin at a concentration of 10 µg/ml and the cellobiase activity is about 1,2075 units/ml.

21. (new) The method of claim 19, wherein the glycosylation inhibitor is 1-deoxynojirimycin at a concentration of about 80 µM and the cellobiase activity is about 1,4085 units/ml.

22. (new) The method of claim 19, wherein the glycosylation inhibitor is glucono-lactone at a concentration of about 2 mg/ml and the cellobiase activity is about 6,1820 units/ml.

23. (new) The method of claim 3, wherein the pH is about 4.5 and the cellobiase activity is about 90 units/ml.

24. (new) The method of claim 3, wherein the pH is about 4.5, the carbon source is mannose, and the cellobiase activity is about 140 units/ml.

25. (new) The method of claim 7, wherein the carbon sources are selected from the group consisting of cellobiose, mannose and succinate.

26. (new) The method of claim 9, wherein the nitrogen source is ammonium dihydrogen orthophosphate.

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27. (new) The method of claim 10, wherein the sterilized medium comprises potato extract.

28. (new) The method of claim 3, wherein the *Termitomyces clypeatus* is a *Termitomyces clypeatus* strain having Indian Institute of Chemical Biology accession number IICB-411.

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